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SYNTHESIS OF DEUTERIUM LABELLED GEOSMIN AND METHYLISOBORNEOL

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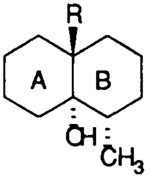
SUMMARY

Geosmin and methylisoborneol have been synthesised with a fully deuterated methyl group for use as internal standards in water analysis.

Key words: geosmin; methylisoborneol; MIB; deuterium

INTRODUCTION

Geosmin 1 and methylisoborneol (MIB) 2 are two highly odorous metabolites of certain algae and bacteria which frequently impart an "earthy" or musty odour to drinking water (1,2). In a previous paper (3) we have demonstrated the benefits of using deuterium labelled analogs of these two compounds as internal standards for determination of geosmin and MIB in water by closed loop stripping analysis followed by gas chromatography/mass spectrometry. We now describe syntheses of the deuterated derivatives: (±)-*trans* -1,10-[$^2H_3$]dimethyl-9α-decalol (geosmin-d_3) and (-)-2-*exo* -hydroxy-2-[$^2H_3$]methylbornane (MIB-d_3). In both cases , the deuterium is introduced via the commercially available Grignard reagent, [$^2H_3$]methylmagnesium iodide.



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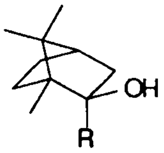
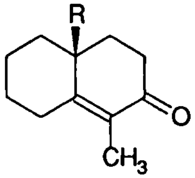


2 R = CH_3
9 R = CD_3



3 R = CH_3

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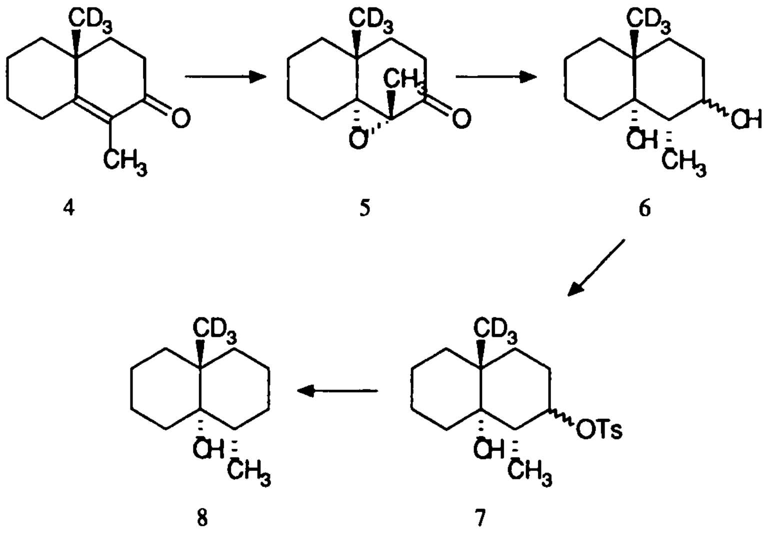
EXPERIMENTAL

(+)-Camphor, 1-chloro-3-pentanone, cyclohexene oxide, methylmagnesium iodide-d_3,m-chloroperbenzoic acid (MCPBA), lithium aluminium hydride (LAH) and lithium aluminium deuteride were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. Infrared spectra were recorded with a Perkin-Elmer 783 spectrophotometer. ^{1}H and ^{13}C spectroscopy was performed on a Jeol FX90Q NMR spectrometer with TMS as internal standard.



2- [$^{2}H_3$]Methylcyclohexanol

Copper (I) iodide (1.91 g; 0.01 mol) was added to a 1 L flask fitted with a Friedrich condenser, two pressure equalising funnels and a magnetic stirrer. The flask was purged with dry argon. Dry THF (100 mL) was added and the stirred mixture cooled to -30 ± 5°C, using an ethanol/dry ice bath. [$^{2}H_3$] Methylmagnesium iodide in ether (0.1 M; 100 mL) was added over 40 min and the mixture maintained at -30°C for a further 10 min. Cyclohexene oxide (6.57 g; 0.067 mol) in 100 mL in THF (100 mL) was added over 10 min at -30°C . The solution was allowed to warm to -5 - 0°C and maintained at that temperature for 2 h. The reaction mixture was poured into saturated ammonium chloride (800 mL) and the organic layer separated. The aqueous layer was extracted with ether (3 x 50 mL) and the combined organic extract washed with sodium thiosulphate (10%; 2 x 50 mL) then brine (3 x 50 mL). The solution was then dried (Na_2SO_4) and the ether removed on a rotary evaporator. The oil was purified by distillation to give 2- [$^{2}H_3$] methylcyclohexanol (9.0 g, b.p. 73°C/ 6 mm) ν max 3340, 2225, 2205, 2127, 2070 cm^{-1} ms m/z Found: 117.124. $C_7H_{11}D_3O$ requires 117.1232.

2- [$^{2}H_3$]Methylcyclohexanone

2-methyl-[$^{2}H_3$]-cyclohexanol (11.4 g) was dissolved in ether (40 ml) in a 250 ml flask in a water bath at 15-20°C. A solution of sodium chromate (10.9 g) and sulphuric acid (7.3 ml) in water (30 ml) was diluted with water to 50 ml and added in small portions over 20-25 min to the stirred solution using a Pasteur pipette. When the addition of oxidant was complete, the mixture was allowed to react for a further 10 min. The phases were separated and the water phase extracted with ether (2 X 10 ml). The combined ether extracts were shaken with saturated sodium bisulphite (50 ml) for 30 min, then washed with 10 ml each of water, 10% sodium bicarbonate, water, brine and then dried (Na_2SO_4). The ether was removed by distillation and the residue distilled through a short Vigreux column at atmospheric pressure to give 2-methylcyclohexanone-d_3 (9.6 g, b.p. 82-85°C / 17mm). ν max (neat) 2220, 2150, 2120, 2070, 1710 cm-1 Mass spectrum: m/z (M+), 115.106 $C_7H_9D_3O$ requires 115.1076.

1,10β-[$^2H_3$]Dimethyldecal-1(9)-en-2-one 4

A mixture of 2-methylcyclohexanone (11.2 g, 0.1 mol), 5-chloro-3-pentanone (18.4 g, 0.15 mol) , p-toluenesulfonic acid monohydrate (600 mg) and benzene was refluxed for 20 h using a Dean Stark apparatus to trap the water produced. The mixture was cooled and diluted with ether (40 ml). The organic layer was washed with saturated sodium bicarbonate (20 ml) and water (40 ml) and the layers separated. The water and bicarbonate extracts were combined and extracted with ether (2 x 20 ml). The ether extracts were combined with the original organic layer and dried (Na_2SO_4). The ether and benzene were removed by distillation and the residual oil distilled under vacuum . The first fraction collected boiled at 85-98°C (bath temperature 150-160 °C) and the second at 98-115°C (bath temperature 160-170°C). The g.c. traces showed 4 to have a purity of 92% and 87% respectively. The two fractions were combined to give 11.5 g of 4 (59 %). ν max (neat liquid) 2220, 2060, 1710, 1665, 1610 cm^{-1}; ^1H n.m.r. ($CDCl_3$) δ 1.76 (s, 3, C-1 CH_3); ^{13}C n.m.r. ($CDCl_3$) δ 10.55, 21.27, 26.63, 27.46, 33.61, 35.75 (C-10), 37.41, 41.80, 120.00 (C-1 or C-9), 128.05 (C-1 or C-9), 162.57 (C-2); ms m/z (M+) 181.156 . $C_{12}H_{15}D_3O$ requires 181.1544.

1β,10β-[$^2H_3$]Dimethyl-1,9-epoxydecal-2-one 5

The enone 4 (5.0 g) was dissolved in dichloromethane (20 ml) and cooled in ice. A suspension of m-chloroperbenzoic acid (8.0 g) in dichloromethane (80 ml) was added over 10 min and the mixture kept 18 h at 4° C. The mixture was shaken with sufficient 10% $NaHSO_3$ to give a negative starch-iodide test, then washed with 5% $NaHCO_3$, 1 M NaOH, water and brine. The solution was dried ($MgSO_4$) and the solvent removed by distillation. The residual oil was purified by distillation under vacuum to give 5 as a colourless oil, b.p. 110-117° C (4.4 g). ν max (neat liquid) 2220, 2160, 2120, 2065, 1755, 1708, 1665 cm^{-1}; ^1H n.m.r. ($CDCl_3$) δ 1.38 (s, 3, C-1 CH_3)^{13}C n.m.r. ($CDCl_3$) δ 11.04, 20.86, 23.90, 26.23, 32.01, 38.03, 65.10 (C-1 or C-9), 71.60 (C-1 or C-9), 207.40 (C-2) ms m/z (M+) 197.149. $C_{12}H_{15}D_3O_2$ requires 197.1493

1α,10β-[$^2H_3$]Dimethyl-2,9α-dihydroxydecalin 6

The epoxide 5 (1.2g) was dissolved in dry dimethoxyethane (5 ml) and treated with LAH (10 ml;

0.5 M in dimethoxyethane). The mixture was refluxed for 2 h, then excess LAH was destroyed with ethyl acetate and the mixture diluted with ether and washed with 1 M HCl, water and brine. The solution was dried ($MgSO_4$) and further dried by azeotropic distillation with benzene. The diol 6 was obtained as a colourless oil (1.16 g) ν max (Nujol) 3400, 2240, 2220, 2175, 2070 cm^{-1}; ^{1}H n.m.r. ($CDCl_3$) δ 0.88 (d, 3, J = 6.3 Hz, C-1 CH_3 , 2.3 (OH), 3.29, 3.44 (C-2 H) ms m/z (M+) 201.181. $C_{12}H_{19}D_3O_2$ requires 201.1806

1α,10β-[$^2H_3$]Dimethyl-9α-hydroxy-2-p-toluenesulphonate 7

The diol 6 (1.16 g) was dissolved in chloroform (10 ml; freed of ethanol by filtration through alumina) and the solution treated with pyridine (1.0 g) and p-toluenesulphonyl chloride (1.44 g). The mixture was kept 14 h at 4° C, then diluted with ether (50 ml) and extracted with 2M HCl, 5% $NaHCO_3$ then brine. The solution was dried ($MgSO_4$) and the ether removed by distillation. The residual oil crystallised on standing at room temperature and the crude 7 was crystallised from ether/hexane to give needles (1.3 g, m.p. 110-111 °C). ν max (Nujol) 3620, 3060, 2240, 2220, 2175, 1597 cm^{-1}; ^{1}H n.m.r. ($CDCl_3$) δ 0.72 (d, 3, J=6.4 Hz, C-1 CH_3), 2.44 (s, 3, Ar-CH_3), 4.60 (m, 1, C-2 H), 7.32 (d, 2, J=8.0 Hz, Ar-H), 7.80 (d, 2, J=8.0 Hz, Ar-H) ^{13}C n.m.r. ($CDCl_3$) δ 9.85, 19.30 (septet, C-10 CD_3), 19.96, 20.43, 27.90, 30.20, 33.14, 34.62, 36.37, 39.99, 75.82 (C-9), 86.02 (C-2), 127.70, 129.59, 134.55, 144.26. ms m/z (M+) 355.191. $C_{19}H_{25}D_3O_4S$ requires 355.1894

(±)-*trans* -1,10β-[$^2H_3$]Dimethyl-9α-decalol (geosmin-d$_3$) 8

The crude tosylate (0.96 g) was dissolved in sodium dried THF and added to a solution of lithium aluminium hydride in 1,2-dimethoxyethane (0.5 M, 20 ml). The mixture was heated at 80 °C under reflux for 3 h, then cooled and treated dropwise with ethyl acetate to destroy unreacted LAH. Water (5 ml) was added and the organic layer diluted with ether (20 ml). Dilute hydrochloric acid (2 M) was added to dissolve the white precipitate of aluminium hydroxide and the mixture shaken. The ether layer was then washed with 2M sodium hydroxide, water and brine and the ether dried ($MgSO_4$). The ether was removed by distillation on a rotary evaporator and the crude 8 was purified by chromatography on silica gel (40 g); elution with ether/hexane (2:98 ; 200 ml) gave geosmin-d$_3$ 8 (0.15 g). ν max (neat) 3620, 3510, 2240, 2220, 2170, 2080 cm^{-1} ; ^{1}H n.m.r. ($CDCl_3$) δ 0.77 (d, 3, J=6 Hz, C-1 CH_3);^{13}C n.m.r. ($CDCl_3$) δ 14.79, 20.74, 20.93, 21.32, 29.85, 30.44, 43.24, 34.92, 35.56, 36.97, 74.27; ms *m/z* 185 (0.4) 167 (0.6) 149 (1.5) 138 (0.8) 129 (8.0) 128 (7.3) 115 (100) 114 (19.7) (M+) 185.185 $C_{12}H_{19}D_3O$ requires 185.1857 .

(-)-2-*exo* -Hydroxy-2-[$^2H_3$]methylbornane

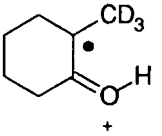
This compound was prepared and purified by the literature method (10) and had m.p. 170° C (sealed tube). ν max (Nujol) 3470, 2220, 2125, 2070 cm^{-1} ; ^{1}H n.m.r.($CDCl_3$) δ 0.84 (s, 3), 0.86 (s, 3), 1.10 (s, 3) ; ^{13}C n.m.r. δ 9.86, 21.12, 21.40, 26.82, 31.29, 45.43, 47.23, 48.89, 51.86, 79.45. ms *m/z* (M+) 171.170 $C_{11}H_{17}D_3O$ requires 171.170.

RESULTS AND DISCUSSION

The synthesis of geosmin-d_3 was based on that of Gosselin et al. (4). The key intermediate is the dimethyldecalenone 3 , which was also used in an earlier synthesis of geosmin by Ayer et al. (5). We prepared a labelled analog of this compound as follows: 2-[$^2H_3$]methylcyclohexanol was prepared by addition of [$^2H_3$]methylmagnesium iodide to cyclohexene oxide in the presence of cuprous iodide catalyst (6). 2-[$^2H_3$]methylcyclohexanol was obtained in 86% yield, but the crude product (after distillation) contained ca. 10% of cyclohexanone. The crude product was oxidised with acidified dichromate by the two phase method of Brown (7) and the resultant 2- [$^2H_3$]methylcyclohexanone was freed of cyclohexanone by extraction with saturated aqueous sodium bisulphite. The 2-[$^2H_3$]methylcyclohexanone was then condensed with 1-chloro-3-pentanone by the literature method (5) with azeotropic removal of water (8) to give, after distillation, a 60% yield of 1, 10β-[$^2H_3$]dimethyldecal-1(9)-en-2-one 4 . This enone was then epoxidised with m-chloroperbenzoic acid , which favours the α epoxide 5 over the β epimer to the extent of 94:6 (4). Our synthesis then departed from that of Gosselin et al. (4), who reduced the keto group of the epoxide with sodium borohydride, tosylated the epimeric mixture of 2° alcohols and reacted both the tosylate and epoxide groups with lithium aluminium hydride . We used lithium aluminium hydride to prepare the diol 6 then selectively tosylated (9) the 2° alcohol to give the monotosylate 7 . This compound crystallised very readily and was more stable than the tosylate epoxide , and despite being a mixture of epimers, had a sharp melting point. The monotosylate 7 was then reduced by addition of a tetrahydrofuran solution of 7 to excess lithium aluminium hydride in 1,2-dimethoxyethane. The racemic geosmin-d_3 8 was purified by chromatography on silica gel.

(-)-MIB-d_3 9 was prepared from (+)-camphor by the literature method (10) and freed of unreacted camphor by conversion of the camphor to its oxime (10). The product is the same enantiomer as MIB derived from algae and bacteria (10).

The mass spectrum of geosmin-d_3 shows a weak molecular ion at m/z 185 but, most importantly, the base peak is at m/z 115 compared with m/z 112 in the natural product. This ensures maximum analytical sensitivity when using the geosmin-d_3 as an internal standard. This suggested that the base peak arose from the ion 10 , formed by loss of ring B as a neutral fragment. This was confirmed by preparing a geosmin-d_5 11 by reducing the epoxide 5 with lithium aluminium deuteride, then converting the diol to geosmin as described above. Geosmin-d_5 gave a molecular ion at m/z 187, but the base peak was still m/z 115.



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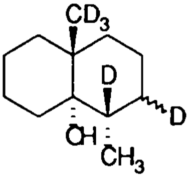

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The MIB-d_3 gave a labelled molecular ion but an unlabelled base peak. This limits the analytical sensitivity, but it is still below the odour threshold. In view of the postulated mass spectral fragmentation for MIB (11), there does not appear to be any simple way to incorporate a deuterium label into the base peak. An attempt was made to label the C-10 methyl group with one deuterium by preparing labelled camphor through the sequence camphene , isobornyl acetate, camphor, MIB. However, when camphene was reacted with D_2SO_4/CD_3COOD, scrambling of the deuterium label in the isobornyl acetate was evident. The gem dimethyl is probably be the most chemically inert site for introducing deuterium, but labelling in this position would require the total synthesis of camphor from [$^2H_6$]dimethylglutaric acid .

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